A new General Purpose Decontamination System for Chemical and Biological Warfare and Terrorism agents

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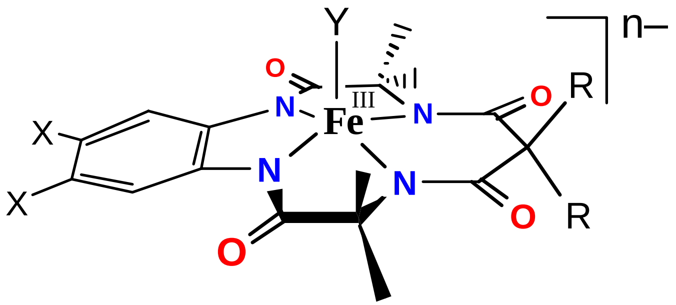
Fe-TAML® Activator of Peroxide



X = H; R = CH_3 FeB*
X = H; R = F $FeBF_2$
X = Cl; R = F $FeDCBF_2$

TAML® Activators developed at Carnegie Mellon University

New Applications

Rapid Inactivation of Bacterial Spores and degradation of organophosphorus triesters as surrogates of Biological and chemical Warfare Agents

'Green' Oxidizing System[1]

- Biomimetic System
- Non-toxic and non-corrosive
- Efficient user of peroxide
- High turnover in oxidative environment

Tested Applications[1,2]

- Effluent Treatment
- Bleaching in Pulp and Paper
- Desulfurization of Diesel
- Dye Transfer Inhibition Agent

1. Collins, T. J. *Accounts of Chemical Research* **2002**, *35*, 782-790
2. http:// www.cmu.edu/Greenchemistry

Activators of Hydrogen peroxide

Relative Rates of Reactive Intermediates Formation

- Bicarbonate Activated Peroxide* System

(Aqueous Foam decon by Sandia National Laboratory)

$$HCO_3^- + H_2O_2 \xrightarrow{k} \mathbf{HCO_4^-} + H_2O \qquad k \approx 10^{-3}\ M^{-1}\ s^{-1}$$

- Fe-TAML® activators of hydrogen peroxide

$$Fe^{III}\text{-TAML} + H_2O_2 \xrightarrow{k'} \text{‘}\mathbf{Fe{=}O}\text{’} \qquad k' \approx 10^{4}\ M^{-1}\ s^{-1}$$

– Deep Oxidation capability

– Non-toxic and non-corrosive

$$\boxed{k'/k = 10^7}$$

* Richardson, D. E. *et al. J. Am. Chem. Soc.* **2000**, *122*, 1729

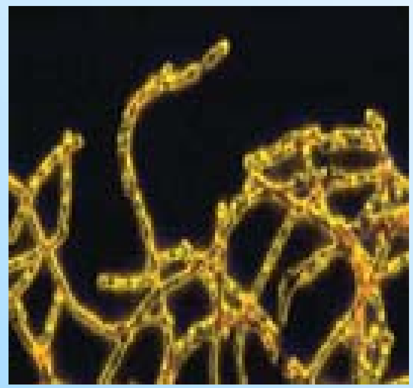
Biological Warfare Agents

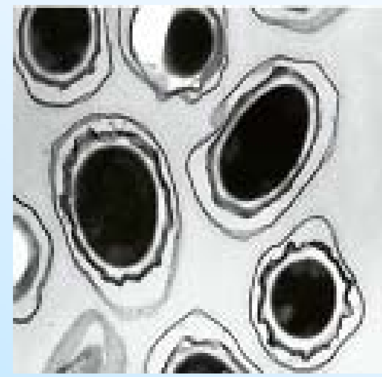
A microorganism or its by-product (toxin), which causes disease in man, plants or deterioration in material; used as weapons of warfare and/or terrorism

Major Threats

- Bacterial Diseases
  - Anthrax
  - Tularemia
  - Plague
- Viral Diseases
  - Smallpox
  - Viral hemorrhagic fevers
- Toxins
  - Botulinum Toxins
  - Ricin

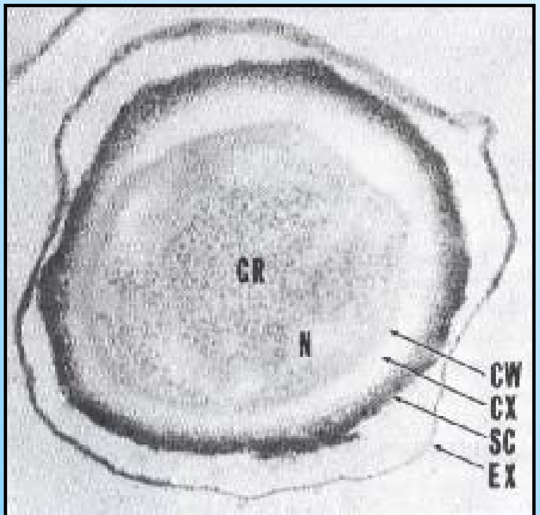
Anthrax Spores



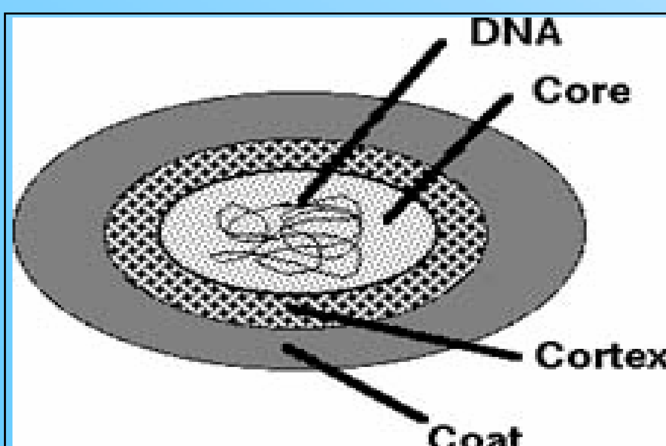


- Dormant survival form of the vegetative bacterium
- Resistant to stress conditions, e.g. heat, UV radiations and chemical treatments
- Germinates on encountering favorable conditions

Bacterial Endospore



Source: L. M. Prescott, Microbiology, McGraw-Hill, NY, 5th Ed., 2002



Spore resistance is due to two protective shells that encase the organism

Spore Coat

Multi-layered highly cross-linked polypeptide structure with numerous disulfide linkages

Spore Cortex

Thick layer of loosely cross-linked peptidoglycan structure with an overall negative charge

Spore Core

- Normal cell structures with ribosomes and a nucleoid
- Metabolically inactive and largely dehydrated

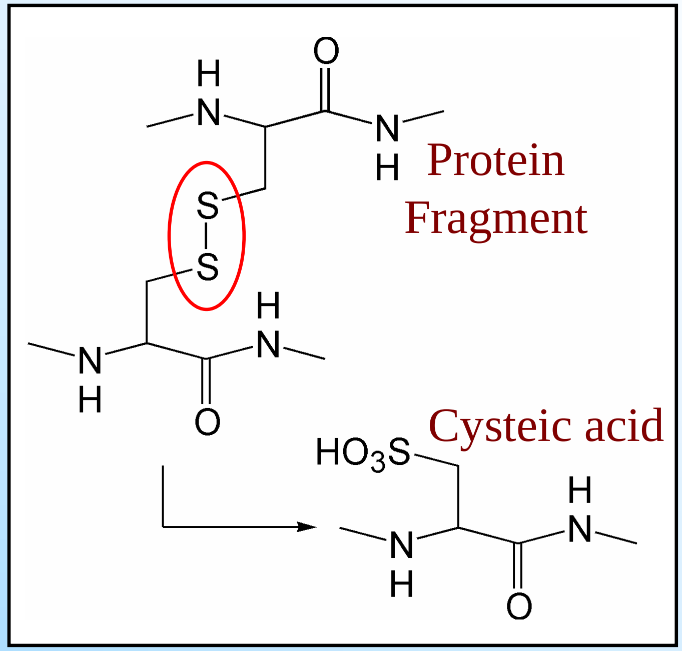
Bacterial Spore Deactivation

Strategies and Mechanism

Strategies

- Penetration of the spore coat with subsequent degradation of bacterial DNA
- Dissolution of spore peptidoglycan structure, exposing the vegetative cell elements
- Initiation of germination with weakening of spore wall followed by deactivation
- Inactivation of spore germination apparatus by destruction of germination-specific lytic enzymes

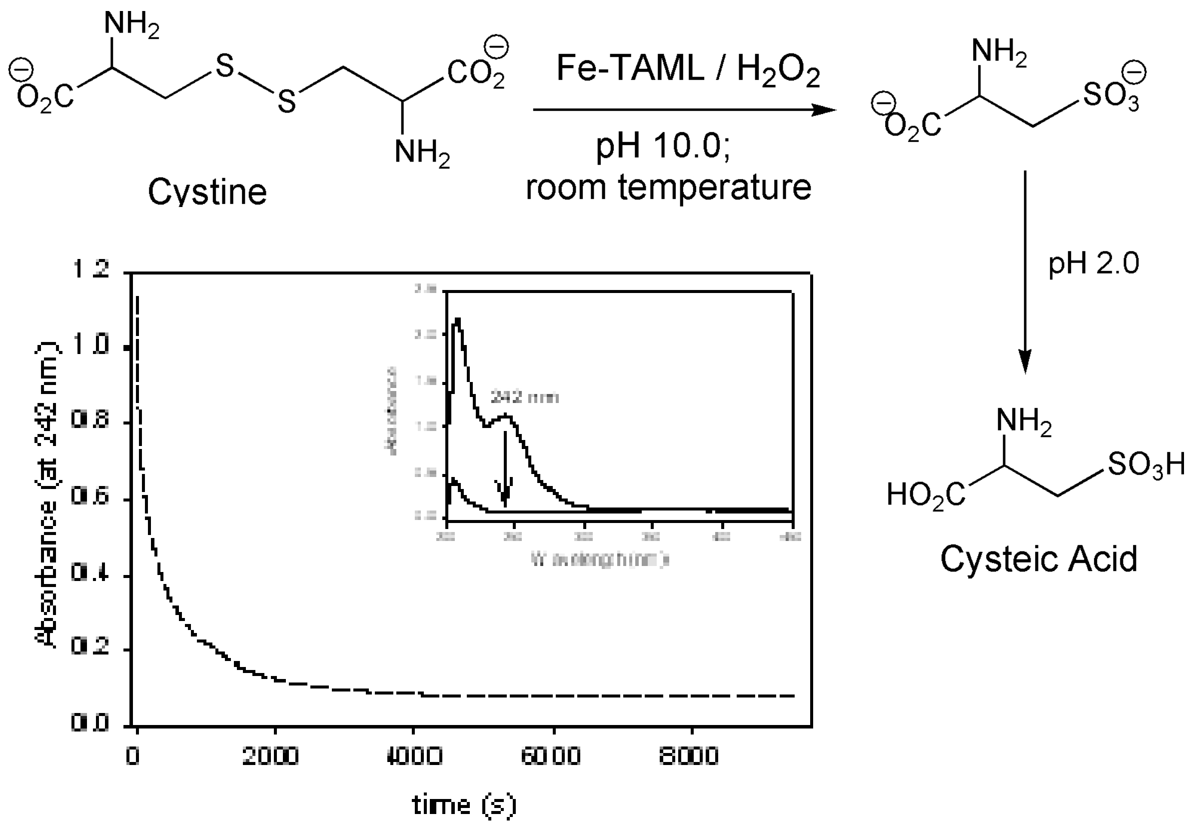
Weakening of spore coat through oxidation of the disulfide bonds



Model compound:
Dialkyldisulfide (e.g. Cystine)

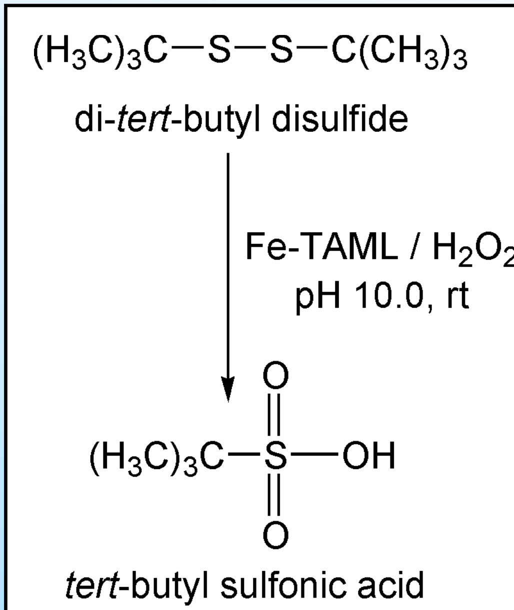
Modeling Studies

Oxidation of Di-alkyl Disulfides



Cysteic acid formation confirmed by HPLC and UV-Visible Spectroscopy

Dissociation of disulfide bonds also observed in di-*tert*-butyl disulfide



Result obtained from ESI-MS studies

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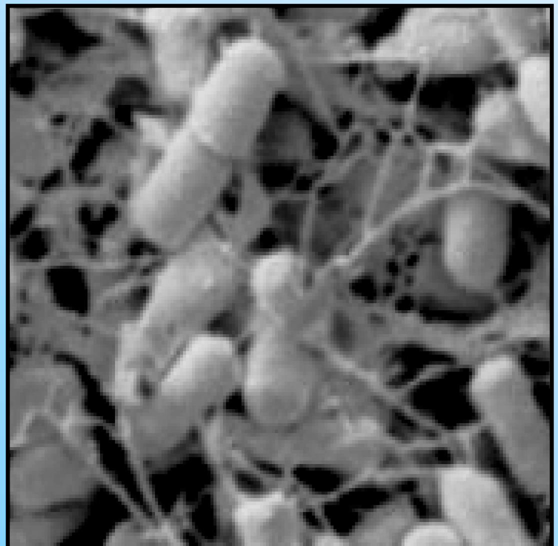
Deactivation Studies with *Bacillus* spores

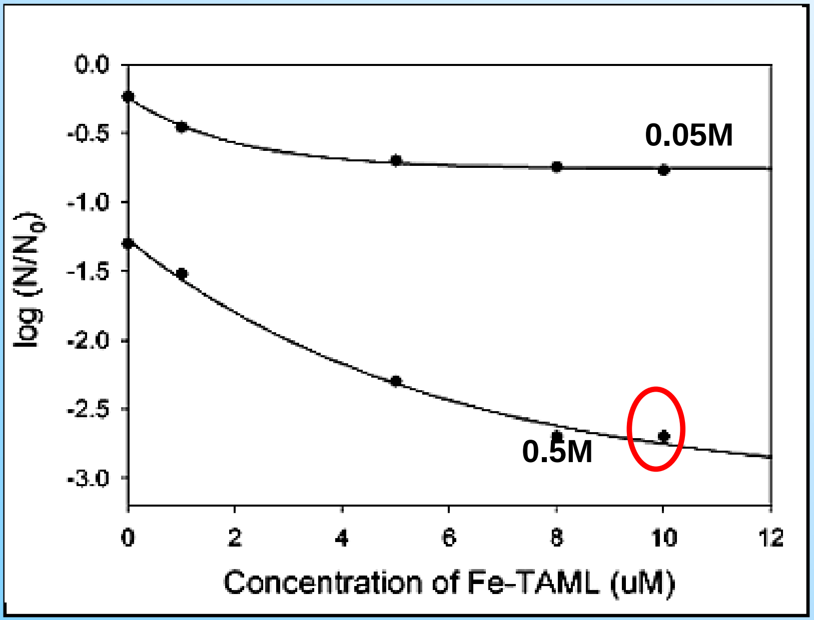


Bacillus atrophaeus
(formerly *B. globigii*)

Spore-forming harmless soil bacterium *B. atrophaeus* (ATCC 9372) was tested as surrogate for *Bacillus anthracis* in spore deactivation studies

Optimization of Reaction Conditions

Variation of Fe-TAML® concentrations



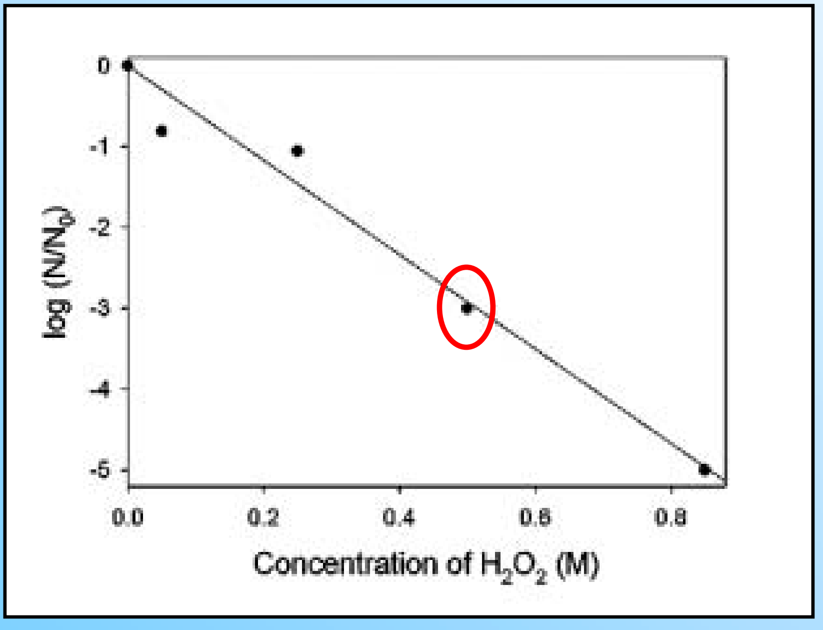
N_0 = Initial number of spores
N = Number of surviving spores

- Studies conducted at two H_2O_2 concentrations
- Exponential relationship between spore deactivation and Fe-TAML® concentration
- Optimized Fe-TAML® concentration: 10 μM

- Reactions carried out for 1 hour at 30°C
- Spore Population of 5×10^7 CFU/ml
- Na-carbonate/bicarbonate (0.1 M) buffer, pH 10.0

Optimization of Reaction Conditions

Variation of H_2O_2 concentrations



N_0 = Initial number of spores
N = Number of surviving spores

- Linear relationship between spore deactivation and concentration of H_2O_2
- Optimized H_2O_2 concentration: 0.5M

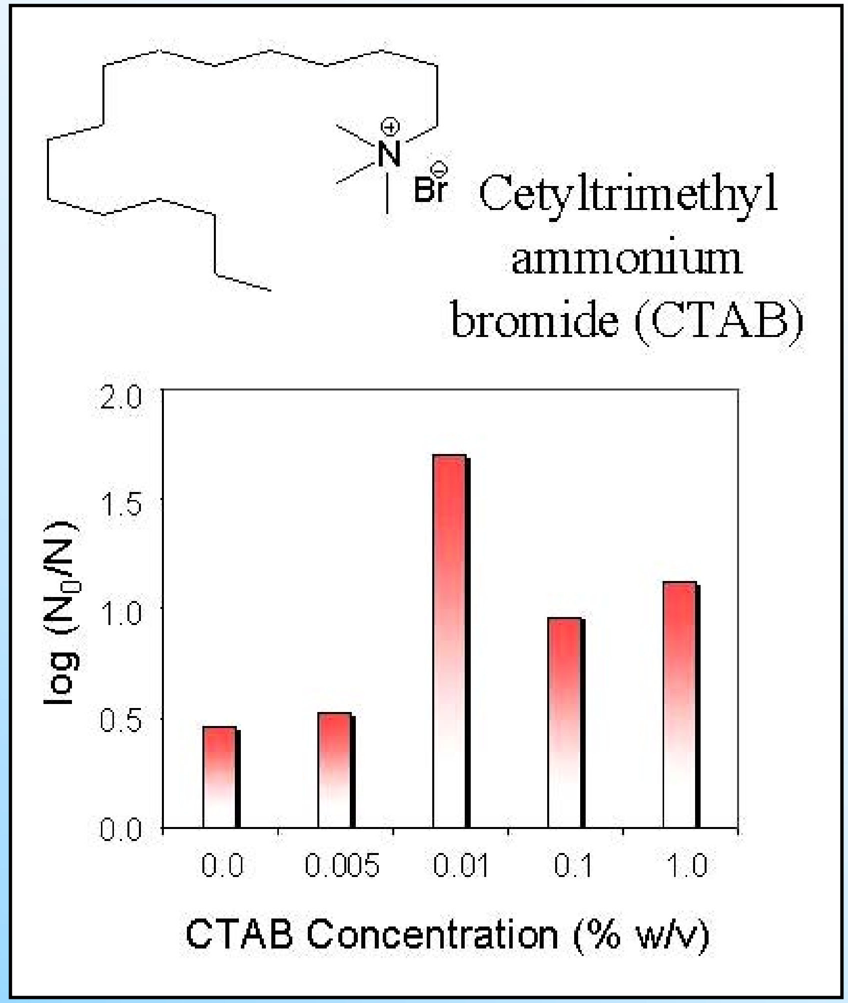
- Reactions carried out for 1 hour at 30°C
- Spore Population of 5×10^7 CFU/ml
- Na-carbonate/bicarbonate (0.1 M) buffer, pH 10.0

Use of Cationic Surfactant

Cationic Surfactants

- Enhance penetrability of Fe-TAML® activators across the spore coat
- Increase dispersion of hydrophobic spores in aqueous phase
- Can cause collapse of spore peptidoglycan structure through ionic interactions

Optimized concentration: 0.03%
(close to cmc value)



N_0 = Initial number of spores
N = Number of surviving spores

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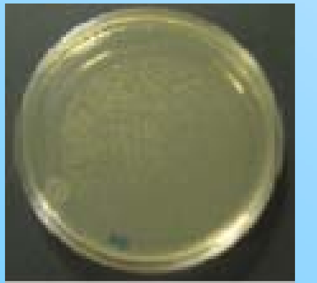
Time Dependence of Spore Kill

10,000×

Control

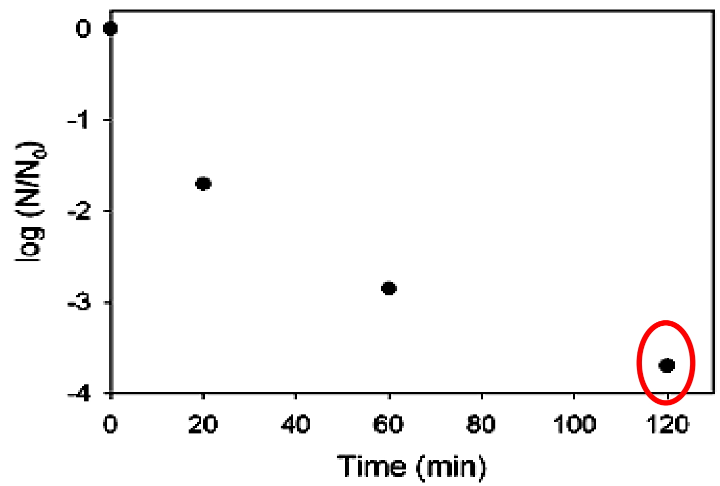
10,000×

95% mortality
with hydrogen peroxide



10,000×

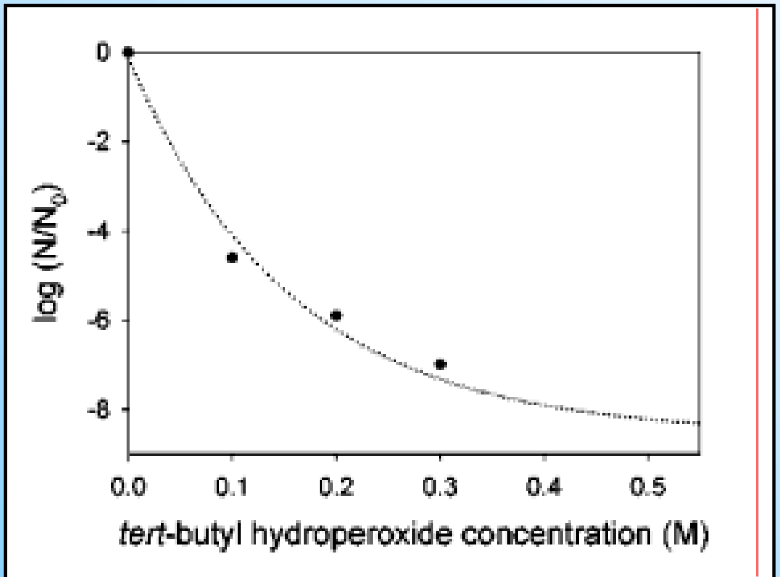
99.98% mortality
with Fe-TAML® activator,
and hydrogen peroxide



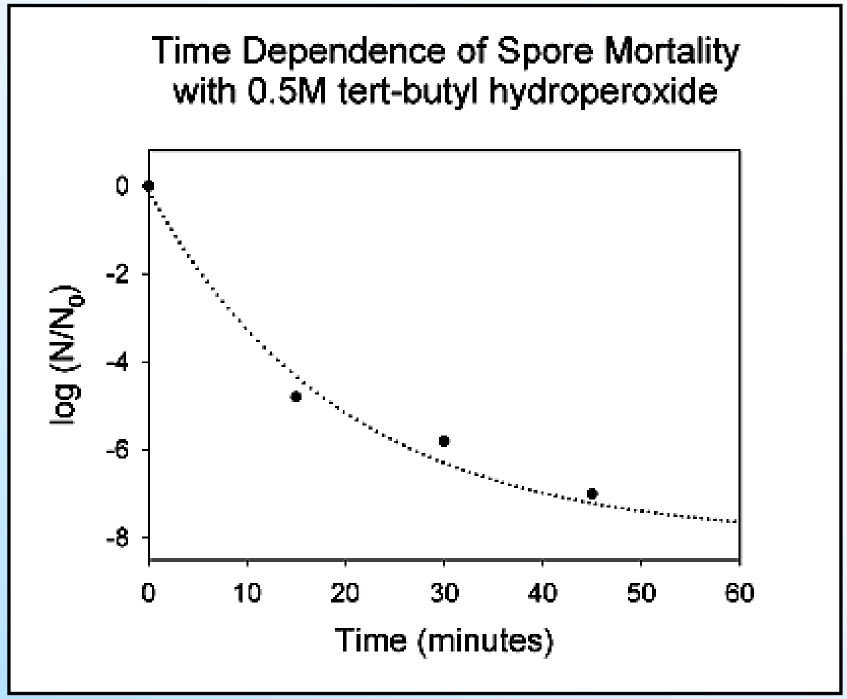
N_0 = Initial number of spores
N = Number of surviving spores

- 99.98% (4-log) kill of spores
- Treatment time: 2 hours
- Fe-TAML®: 10 µM; H_2O_2: 0.5 M
- Spore population: 1×10^8 cfu/ml

Enhanced Spore Mortality



N_0 = Initial number of spores
N = Number of surviving spores



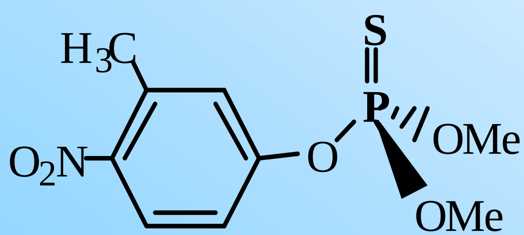
75% with ^{t}BuOOH

99.99999% with Fe-TAML® + ^{t}BuOOH

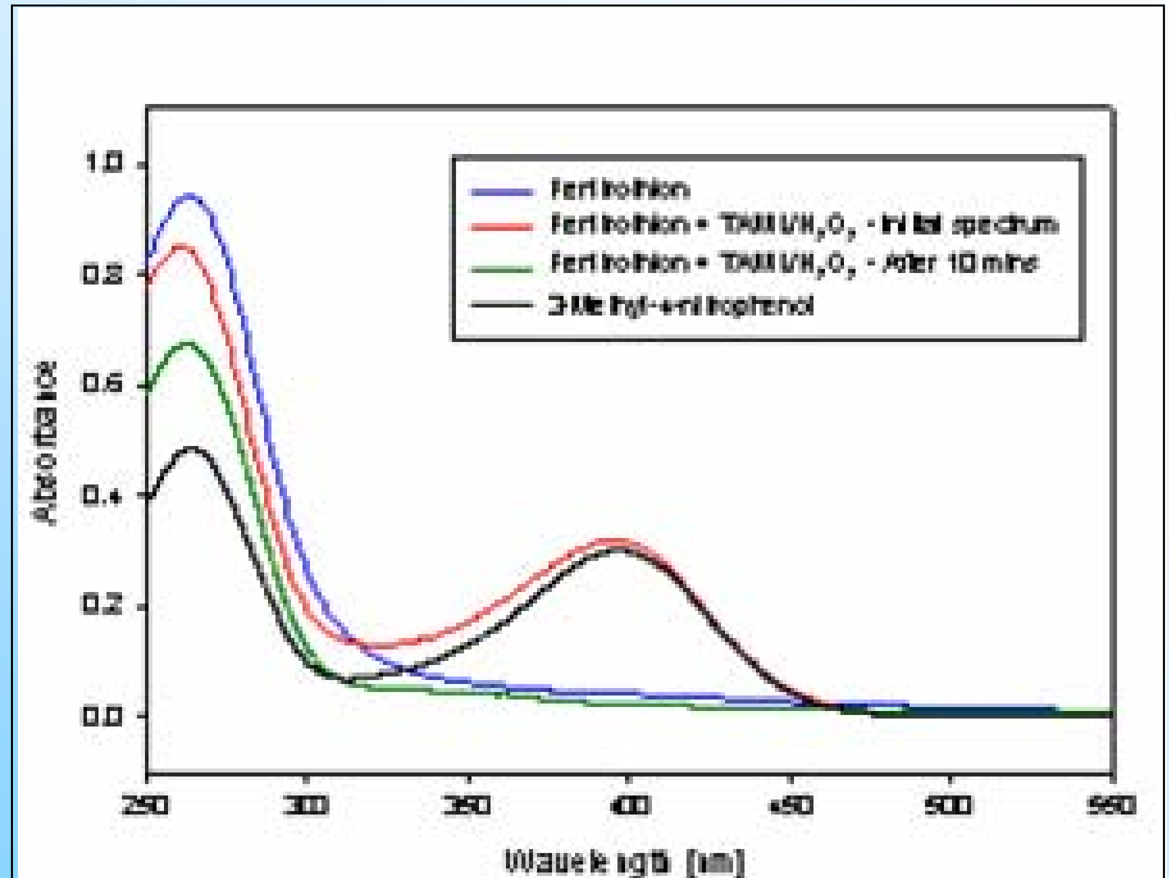
- 99.99999% (7-log) kill of spores
- Treatment time: 1 hour
- Fe-TAML®: 5 μM; ^{t}BuOOH: 0.3 M
- Spore population: 1×10^8 cfu/ml

Oxidative Detoxification of Organophosphorus Triesters and Dialkyl sulfides

TAML®-activated H_2O_2 Treatment of Fenitrothion

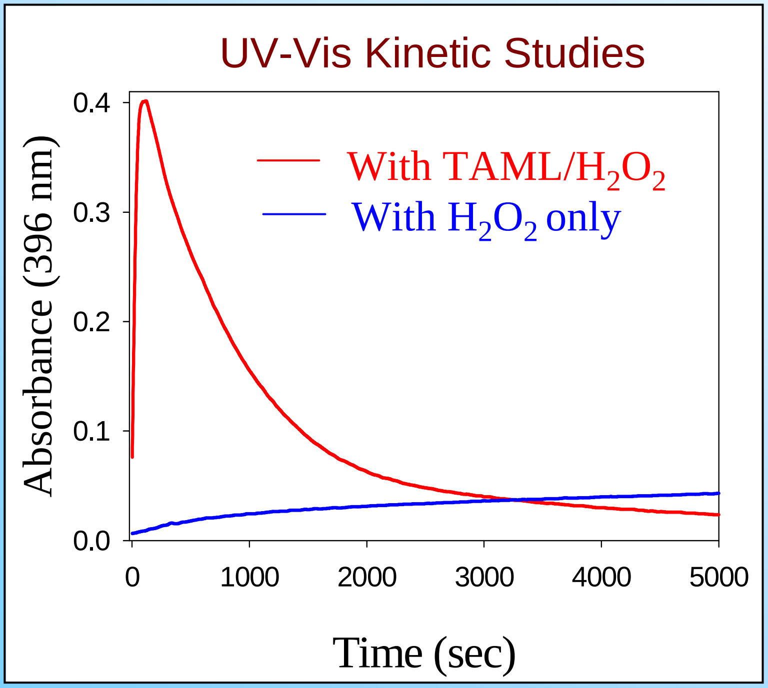


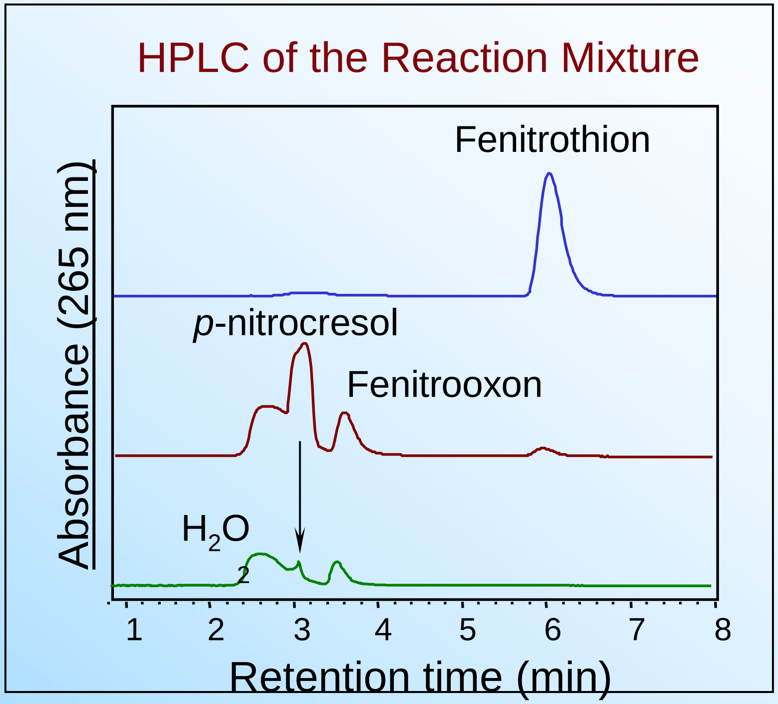
Fenitrothion



UV-Visible spectroscopic study

TAML®-activated H_2O_2 Treatment of Fenitrothion





Kinetics of decomposition of fenitrothion is followed through absorption at 396 nm In UV-Vis. Rapid hydrolysis is seen followed by degradation of *p*-nitrocresol.

Time-lapsed analysis of the reaction mixture by HPLC shows initial formation of *p*-nitrocresol and fenitrooxon. In subsequent stage, most of *p*-nitrocresol is degraded.

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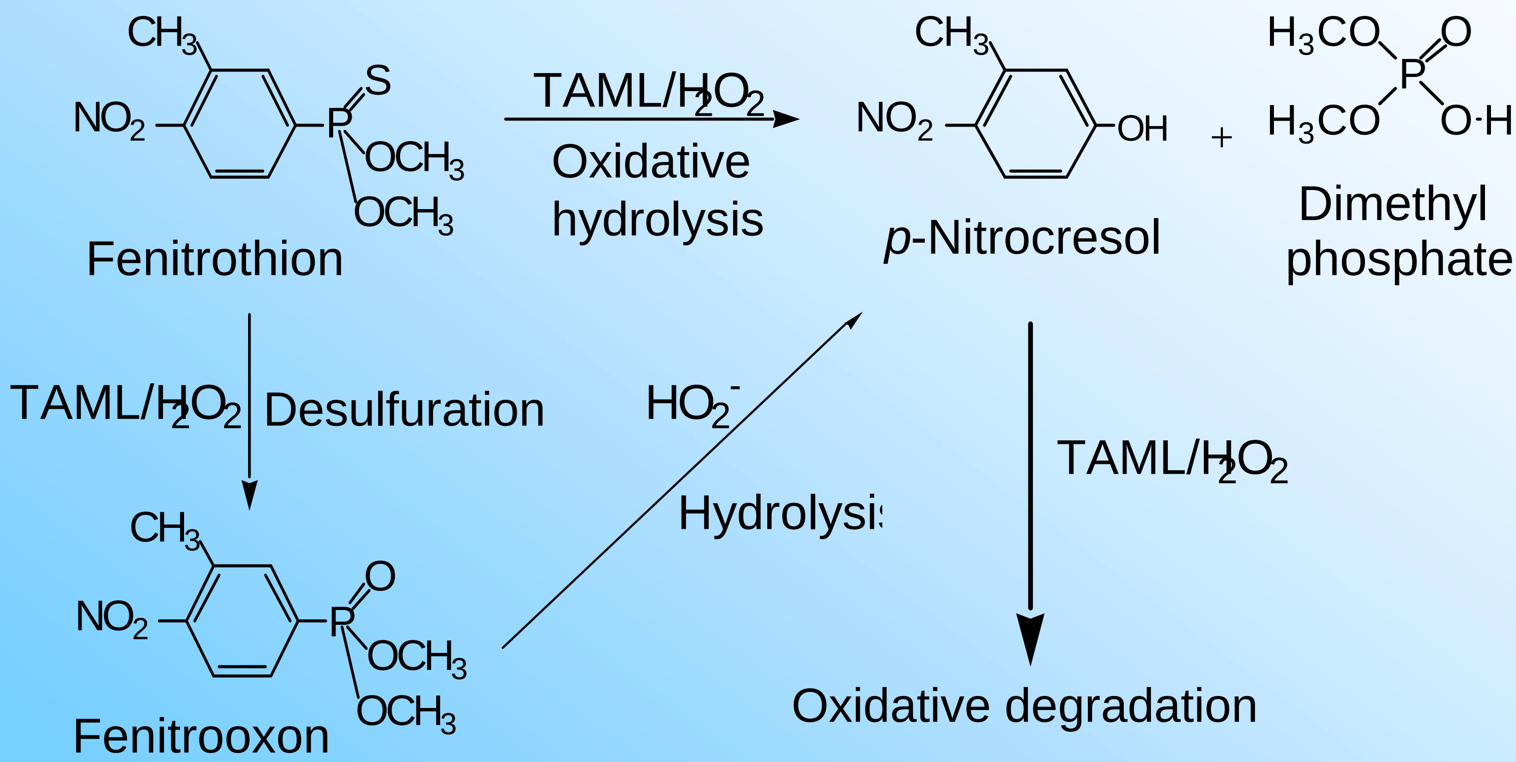
TAML-activated peroxide decomposition of Fenitrothion



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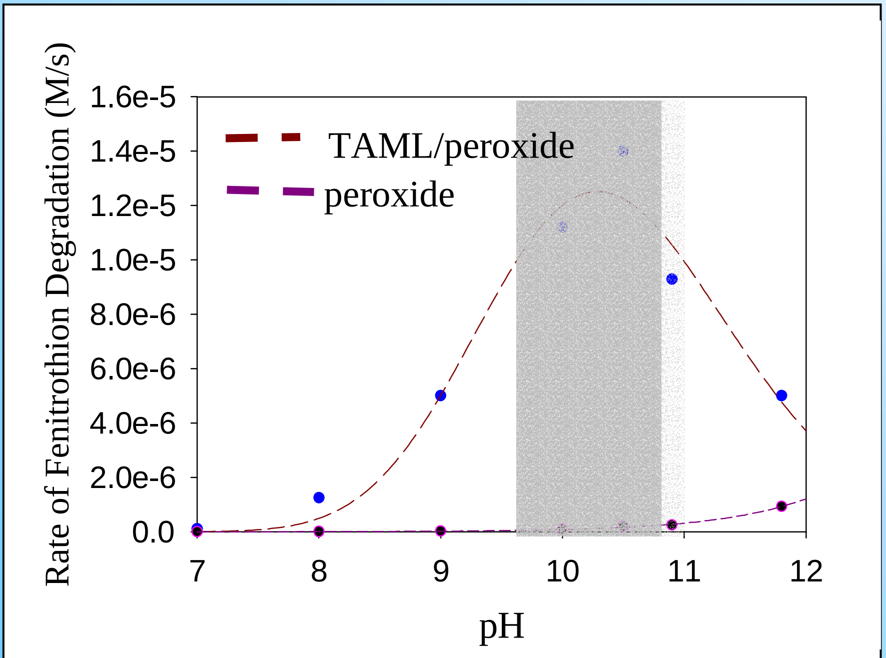
Fenitrothion and Fenitrooxon Degradation - pH Dependence

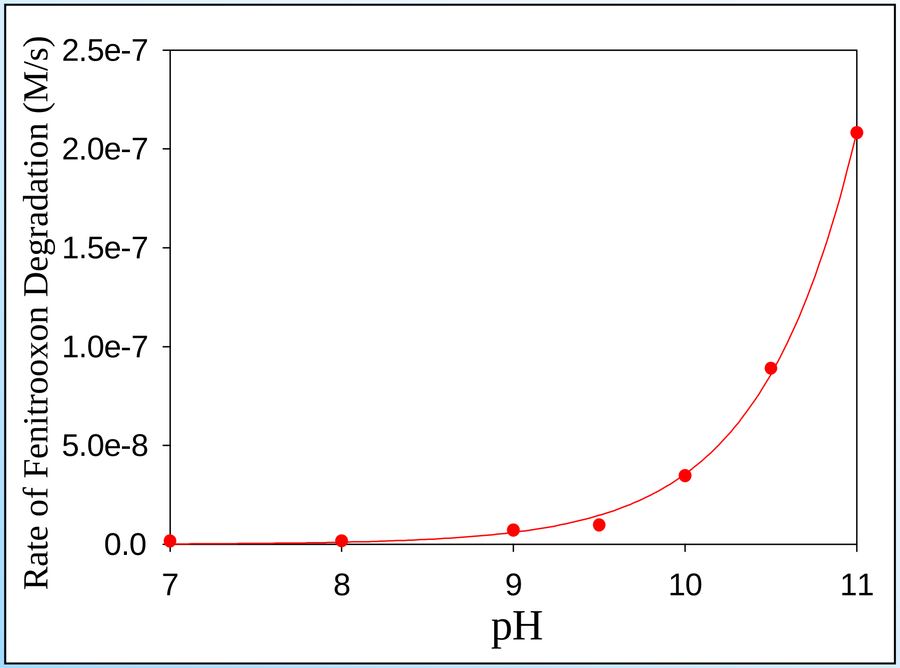
Initial rate measurements following *p*-nitrocresol formation (395 nm)

TAML/H_2O_2 mediated Fenitrothion degradation



TAML®: Fenitrothion: peroxide (1: 25: 50,000) in phosphate buffer (0.1M)

Peroxide assisted Fenitrooxon degradation



Fenitrooxon: peroxide (1:2000) in phosphate buffer (0.1M)

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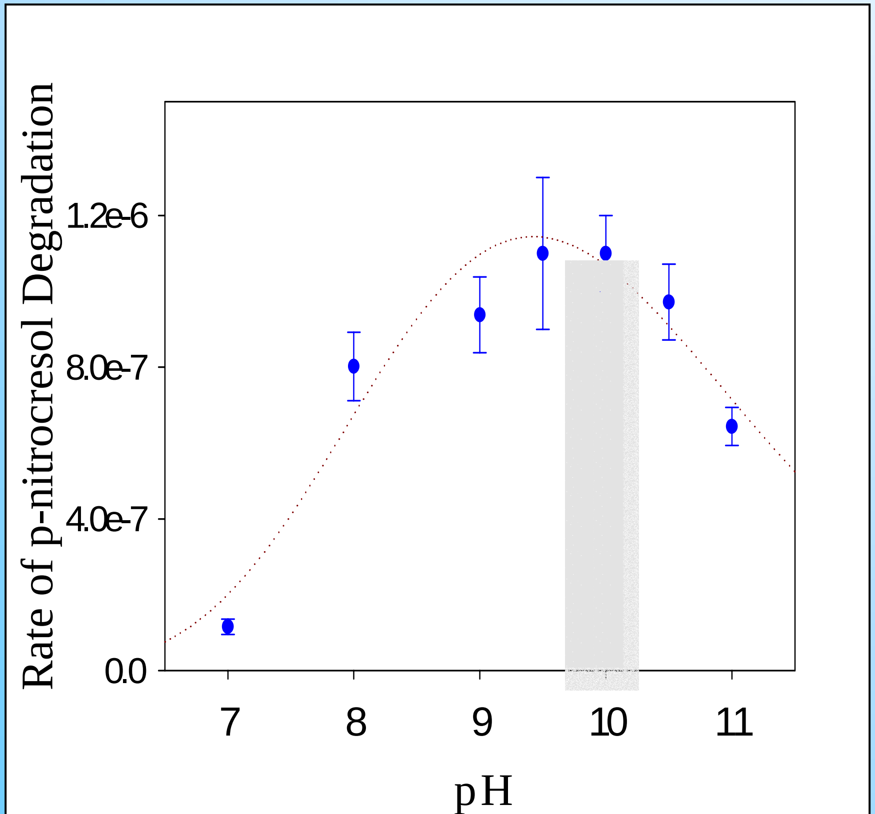
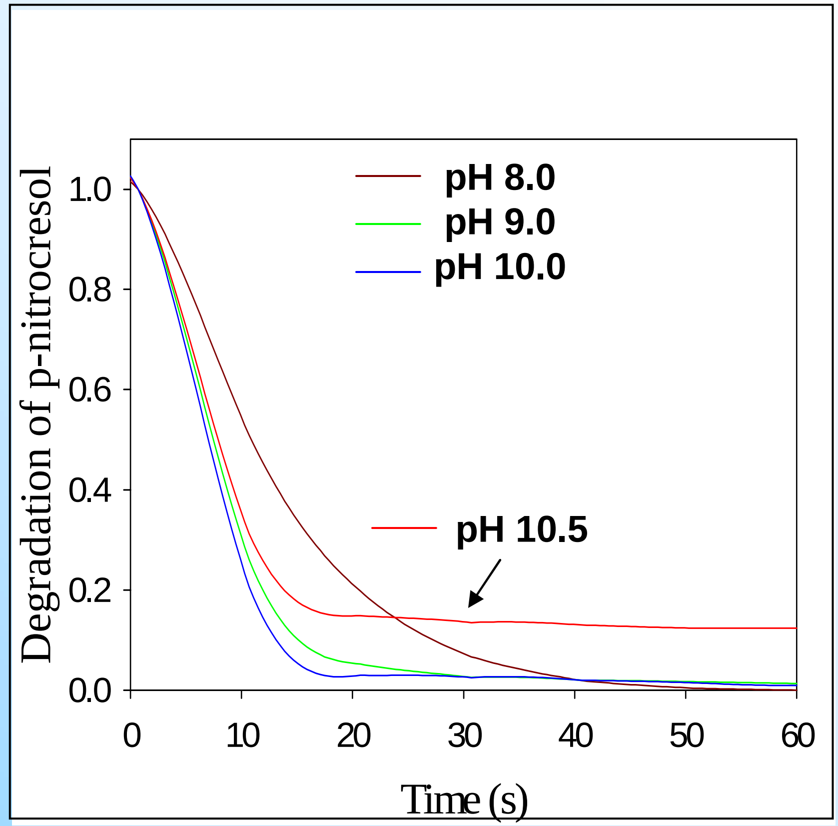
p-Nitrocresol Degradation – pH dependence

Optimization of Reaction Conditions

Initial rate measurements



Kinetics of oxidative degradation



At higher pH, the reaction rate increases, but catalyst gets inactivated faster

Optimum pH range 9.5-10.0

Summary of Fenitrothion degradation Study

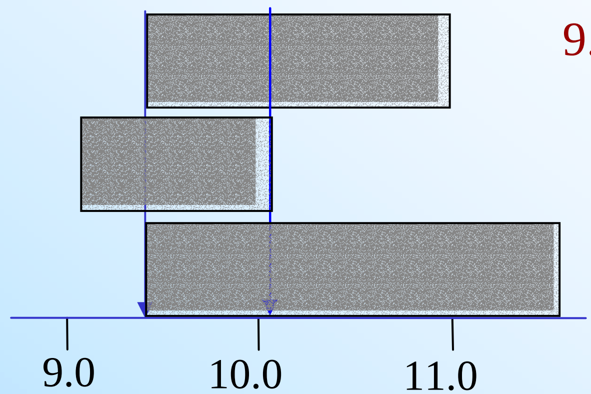
Optimal pH

Limiting condition

Fenitrothion – Oxidative hydrolysis — 9.0-11.0

p-Nitrocresol – Oxidative degradation — <10.5

Fenitrooxon – Peroxy anion assisted hydrolysis — >9.5



Optimal reaction conditions for Total degradation of fenitrothion

pH 9.5-10.0, phosphate buffer (0.1 M), 25°C

TAML®: Fenitrothion: Peroxide 1 : 25 : 50,000

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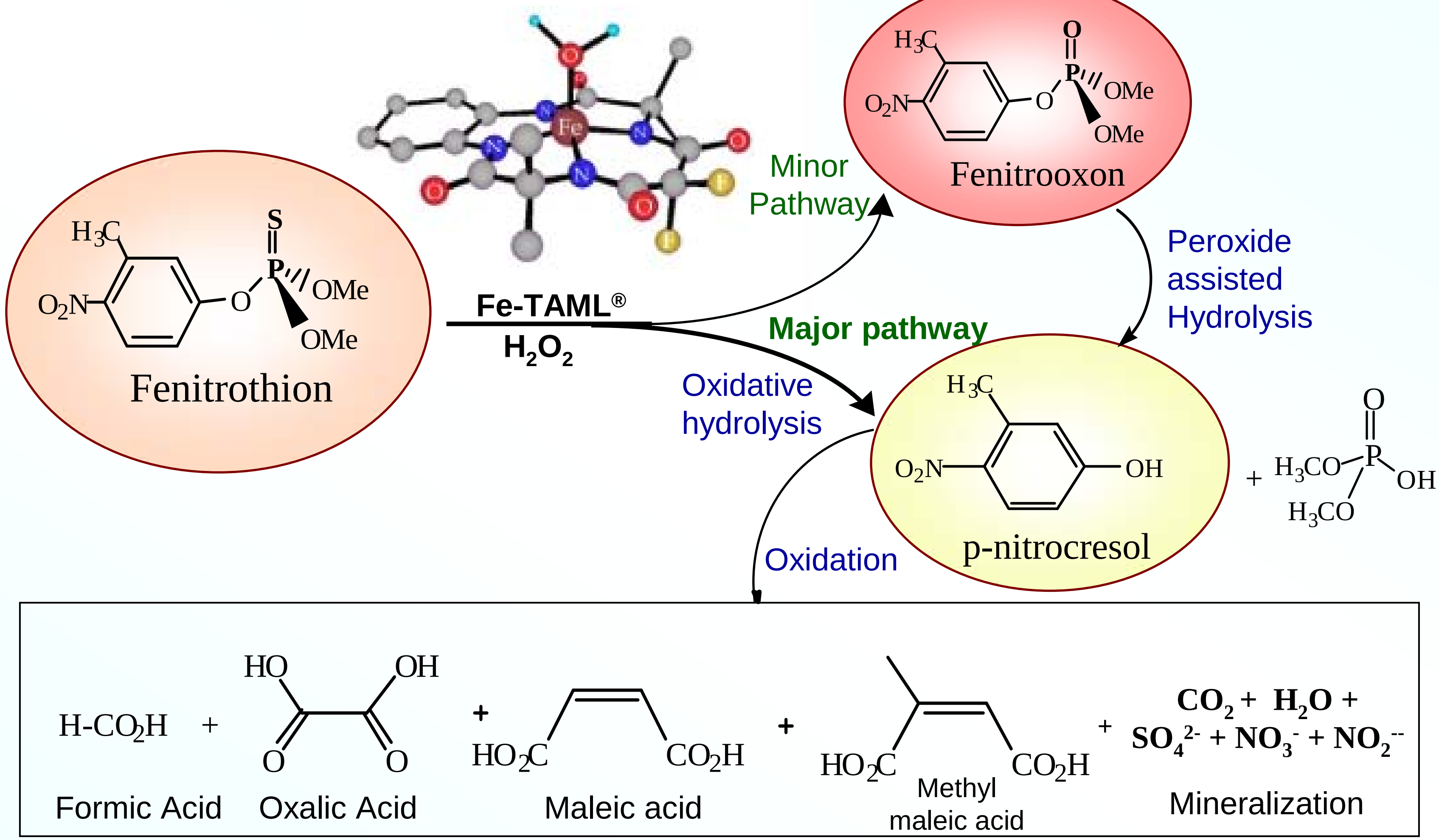
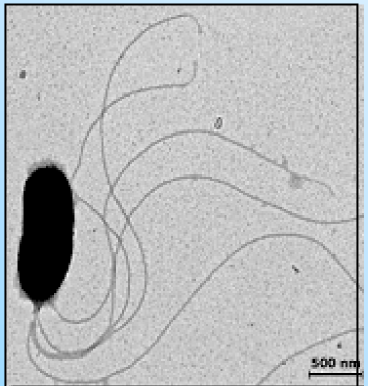
Total Degradation of Fenitrothion



Fenitrothion Degradation

Aquatic Toxicity



Vibrio fischeri

Dafnia Magna

	MicroTox EC_{50} (15 min.) Mg/L	*D. Magna* EC_{50} Mg/L
Fenitrothion (99%)	2.33	14.1
TAML catalyst ($FeBF_2$)	58.00	NA
Reaction mixture (pH 10, quenched with *catalase*)	57.25	>530
Reduction in toxicity	25-fold	>38-fold

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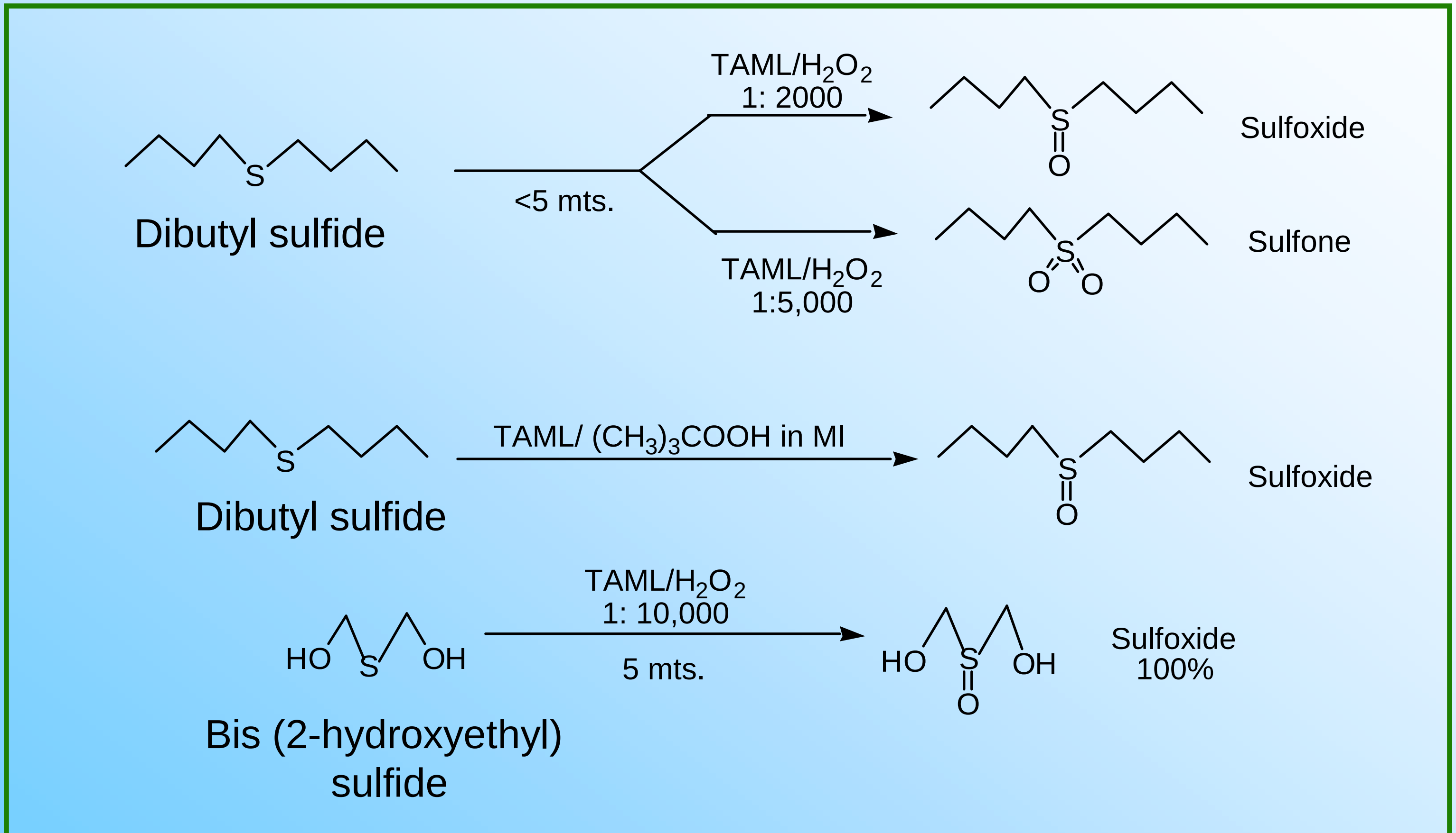
Reaction of Dialkyl sulfides with TAML/peroxide



TAML:substrate = 1:1,000; pH 8; Phosphate buffer, 25°C

Conclusions

TAML®-peroxide technology:

- Effectively deactivate bacterial spores, the toughest of all microorganisms, in aqueous solution achieving 99.99999% (7-log) of spore destruction
- Rapidly detoxify organo-phosphorus triesters, followed by the deep oxidation of hydrolysates
- Selectively oxidize dialkyl sulfides to less toxic sufoxide
- Promises an environmentally friendlier superior technology for destruction of all chemical-biological warfare agents

New Decon System Features

- **Catalytic** – Requires very low catalyst and low peroxide concentration
- **Designed to be Non-toxic** – No toxic elements or functionality
- **Aqueous based** – Compatible with wide variety of surfaces and technologies; can be used on sensitive equipment
- **Broad-spectrum activity** – Detoxify and degrade large-range of chemicals and inactivate bacterial spores
- **Performance previously unavailable** – Truly biomimetic with deep oxidation capability (leaves no toxic biproducts)
- **Robust system** – Stable and functional over wide range of pH
- **Rapid acting and safe** - for people and environment
- **Easy to use** – Used at ambient conditions, offers a practical approach

Acknowledgements

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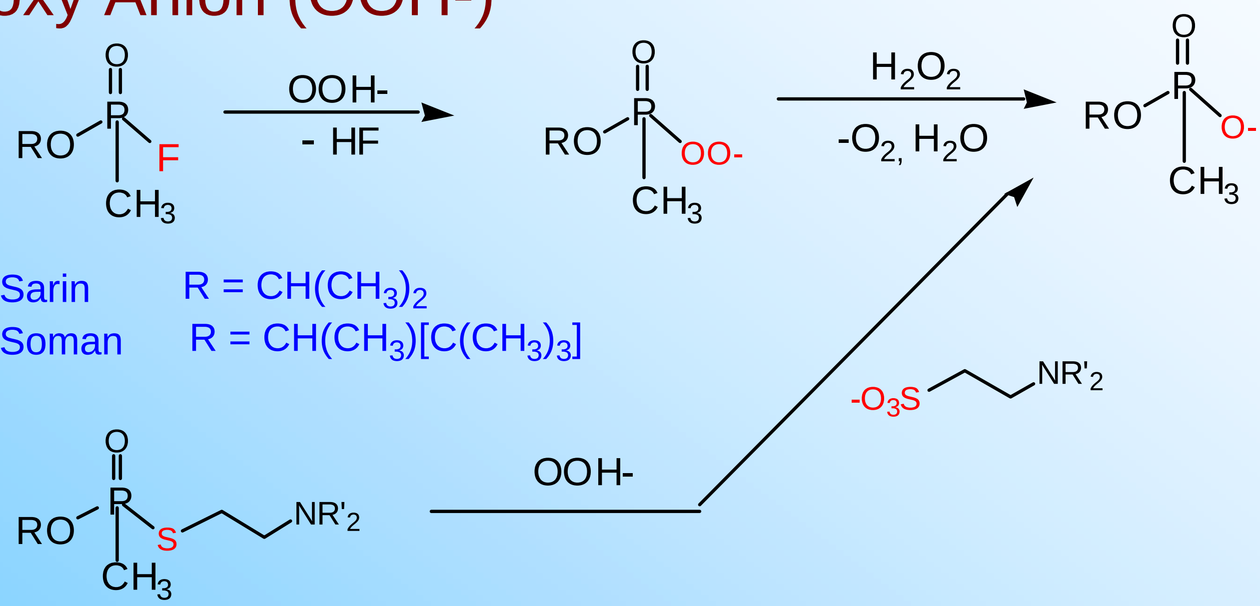
DURIP

Nucleophile assisted Hydrolytic Detoxification of Chemical Warfare Agents

Peroxy Anion (OOH-)



Sarin $R = CH(CH_3)_2$

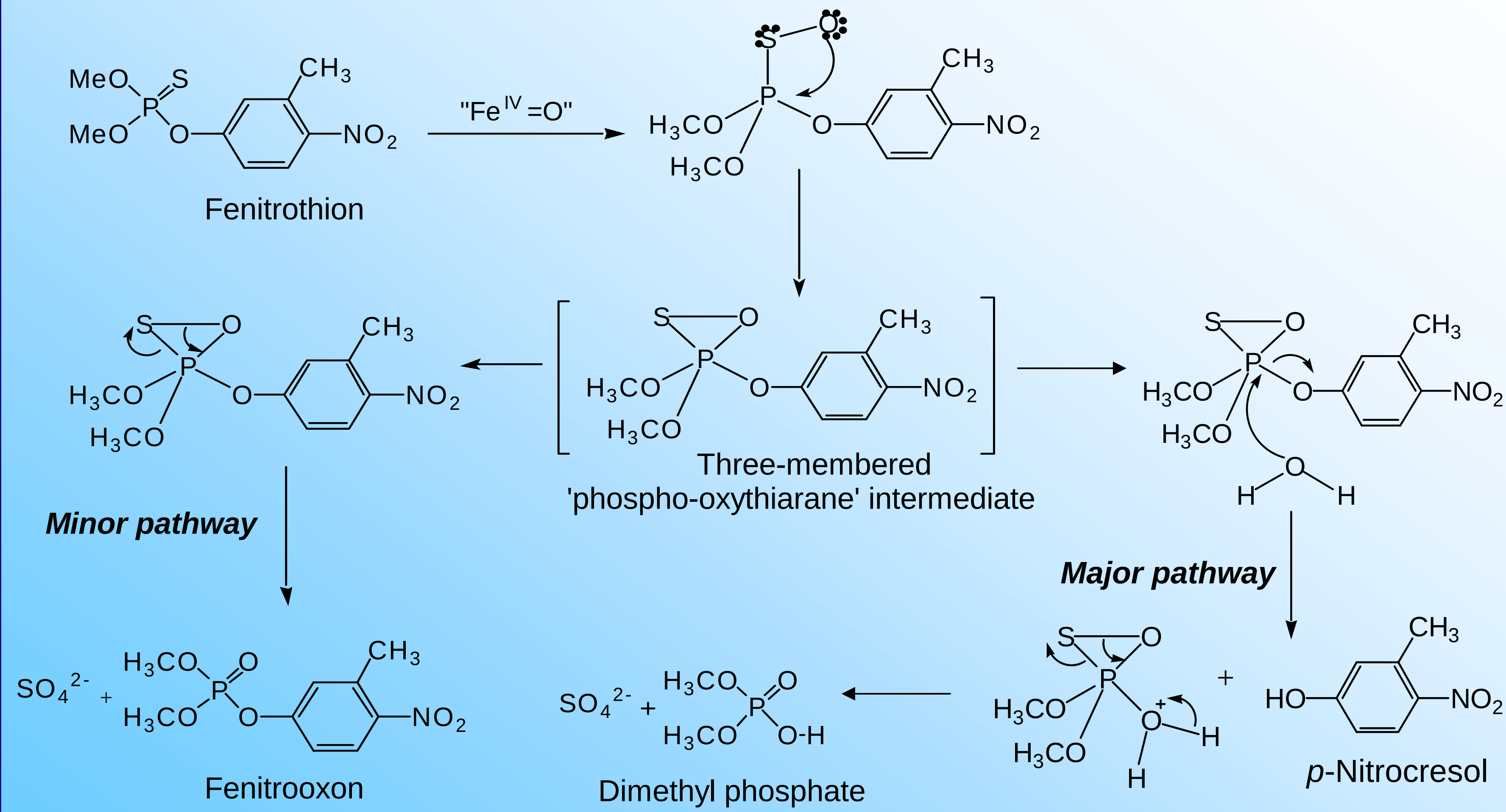
Soman $R = CH(CH_3)[C(CH_3)_3]$

VX $R = CH_2CH_3$ $R' = CH(CH_3)_2$

The rate of nucleophile aided hydrolysis of esters is increased by cationic micelles (e.g. $^{-}$OOH/CTABr).

Wagner and Yang, 2002.
Ind. Eng. Chem. Res., 41(8), 1925-1928

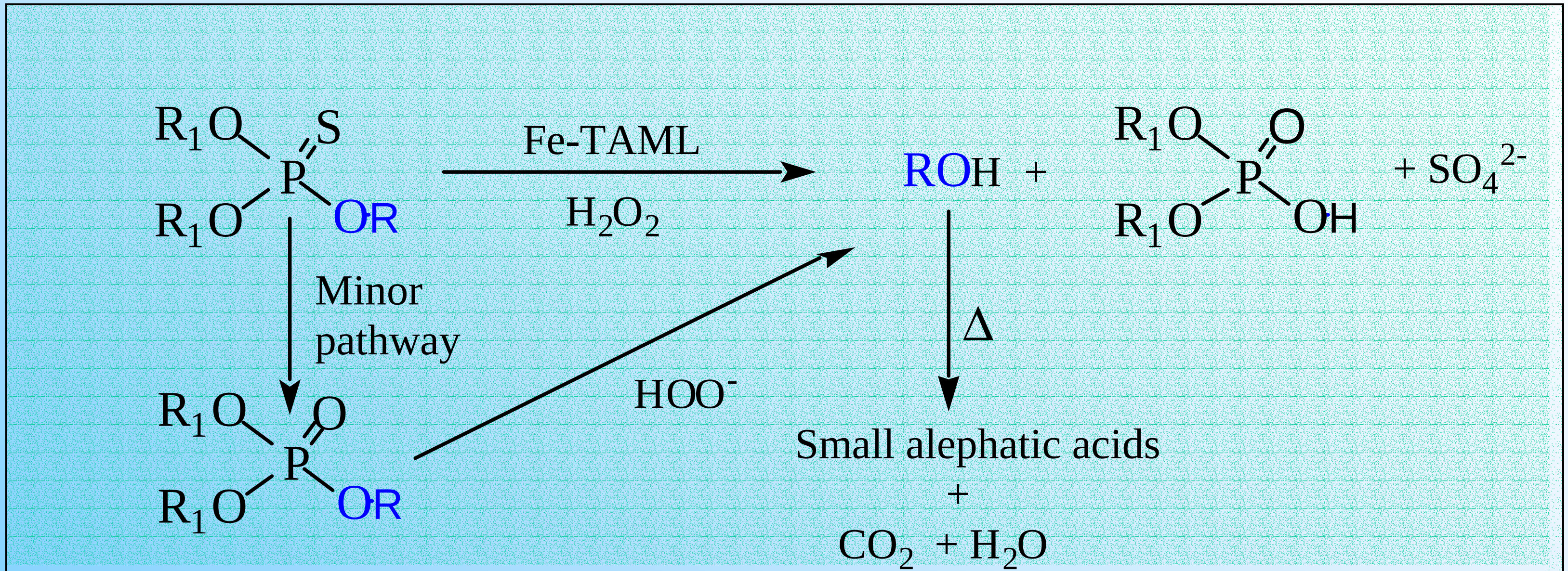
Fe-TAML peroxide oxidant system mimics Cytochrome 450



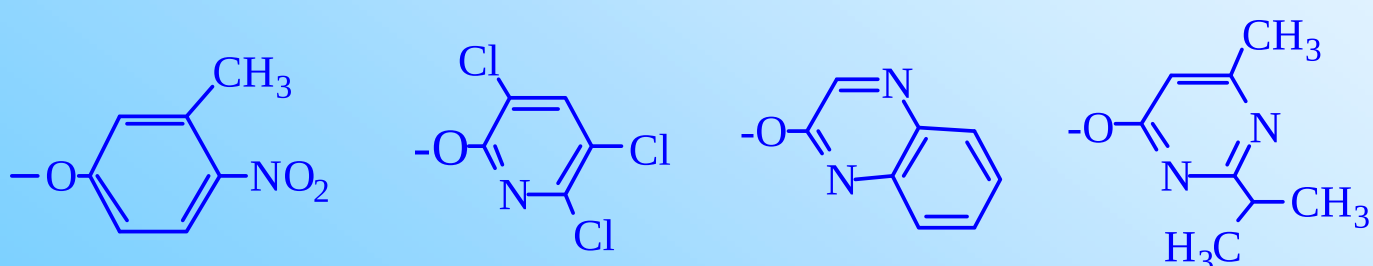
TAML-activated peroxide treatment of fenitrothion possibly results in a common 3-membered ring intermediate formation leading to fenitrooxon and p-nitrocresol

TAML/H_2O_2 Degradation of Organophosphorus Triesters

A Versatile and Robust Process



R =



Fenitrothion Chlorpyriphos Quinalphos Diazinon

R_1 = $-CH_3$, $-C_2H_5$

Catalysis of Phosphate Triester Hydrolysis by Cationic Micelles

- Nucleophile (such as peroxide anion) aided hydrolysis is the most preferred reaction to detoxify phosphorus esters.
- The rate of nucleophile aided hydrolysis of esters is increased by cationic micelles (e.g. $^{-}OOH/CTABr$).[1,2]
- CTABr has significantly enhanced hydrolytic rate of phosphorus esters, (depending on substrate, 20-300 fold enhancement) with hypochlorite.[1]
- Aqueous cationic micelles accelerate spontaneous hydrolysis of dinitrophenyl phosphate and acyl phosphate dianions, with an extensive P-O bond cleavage in the transition state.[3]

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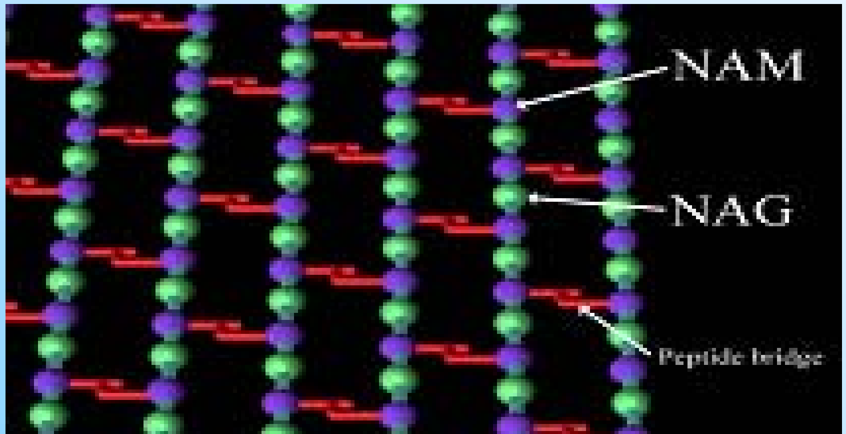
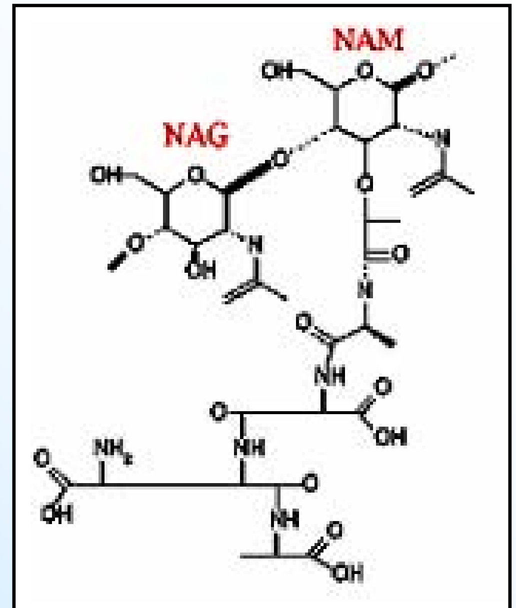
1. Dubey, Gupta et al., *Langmuir*, **2002**, 18, 10489-10492
2. Couderc and Toullec, *Lanmuir*, **2001**, 17, 3819-3828.
3. Brinchi, profio et al., *Langmuir*, **2000**, 16, 10101-10105

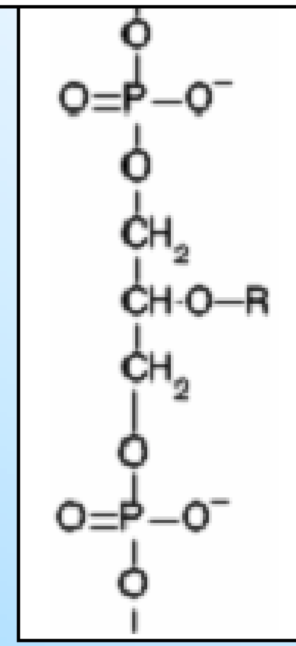
Bacterial Endospore

Spore Cortex





- Loosely cross-linked peptidoglycan composed of *N*-acetyl glucosamine and *N*-acetylmuramic acid with short peptide side-chains
- Maintains spore dormancy and heat resistance; hydrolyzes during germination
- An overall negative charge — from the phosphate backbone of teichoic acid (20-40% of dry weight of cortex)



Teichoic Acid